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Association between chronic hepatitis C infection and hepatocellular carcinoma in a Scottish population

G H Haydon, L M Jarvis, P Simmonds, D J Harrison, O J Garden, P C Hayes

Abstract

Background—Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. The geographical prevalence varies considerably in different countries and Scotland is regarded as an area of low risk for the disease.

Aims—To assess the association between chronic hepatitis C infection (HCV) and HCC in a population of patients presenting to a single hospital.

Patients—One hundred and fourteen cases of histologically confirmed liver cancer presenting to the Royal Infirmary of Edinburgh between 1985 and 1994 were examined.

Methods—Of 114 cases of HCC, 80 samples of stored sera were available. Samples positive for HCV Ab were genotyped by restriction fragment length polymorphism analysis of HCV c-DNA. A population of 29 cirrhotic patients (diagnosed between 1985 and 1994) with chronic HCV infection was also genotyped.

Results—Chronic HCV infection was a major risk factor (30% of tested HCC patients) identified. HCV genotype 1b was predominant (16 of 20 patients). The time from HCV transmission to development of cancer ranged from 10 to 50 years (median 30). In the cirrhotic patient population, a broader distribution of genotypes was present (genotype 1a: 7; genotype 1b: 8; genotype 2b: 3; genotype 3a: 8 and genotype 4: 2). However, this population was significantly younger. (Mean (SD) 52 (14.5) years) ($p=0.0002$) and demonstrated a significantly shorter duration of infection: range 10–40 years (median: 19).

Conclusion—There is a strong association between chronic HCV infection, cirrhosis, and hepatocarcinogenesis in this Scottish population. The study was unable to distinguish whether the high prevalence of genotype 1b in the HCC population reflected increased oncogenicity in itself, or whether 1b was simply the most prevalent genotype in Scotland when these patients were infected.

(Gut 1997; 40: 128–132)

Keywords: hepatocellular carcinoma, hepatitis C virus, HCV genotypes, hepatitis B virus.

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world, with an estimated annual incidence of 500 000 to

1 000 000 new cases.¹ Geographical areas carry different risk factors; the United Kingdom is regarded as an area of low risk of developing the disease.² It has been known for many years that hepatitis B virus (HBV) infection, aflatoxin B1 exposure, consumption of excess alcohol, and some hereditary conditions (haemochromatosis, α_1 antitrypsin deficiency, and hereditary tyrosinaemia) are important risk factors. Recently, there has been renewed interest in primary liver cancer, following the recognition of the importance of chronic hepatitis C infection (HCV); epidemiological surveys have identified HCV in between 15–80% of patients with HCC depending on the population studied.³ HCV seems to be the major cause of HCC in Japan, Italy, and Spain, but is less important in South Africa and Taiwan.^{4–12} There are no published data relating to HCV associated HCC in the United Kingdom.

Examination of the epidemiology of HCV infection in different geographical regions and in different age, risk, and racial groups has become possible following the identification and classification of viral genotypes.¹³ The viral genome is divided into a structural and non-structural region. The structural region contains genes for a nucleocapsid protein and the envelope glycoproteins. The genes in the non-structural region code for functional proteins. In addition, a nucleotide sequence at the 5' portion in the structural region does not encode a gene product. This 5' non-coding (5'-NCR) sequence is the most highly conserved region; the greatest heterogeneity occurs in the envelope proteins. HCV has been classified into a total of six major genotypes, showing not more than 70% sequence homology, by the distinct nucleotide sequences in the 5'-NCR; many of these contain a number of more closely related subgroups (a, b, and c).¹⁴ Viruses may be typed by restriction fragment length polymorphism (RFLP) analysis of cDNA amplified from the 5'-NCR by the reverse transcription polymerase chain reaction (RT-PCR).¹⁵ Preliminary reports suggest major biological differences between the genotypes in response to treatment with interferon^{16–18} and perhaps, progression and severity of disease.¹⁹

In this study, we aimed to assess the association between chronic HCV infection and HCC over a 10 year period, in cases presenting to a single hospital in Scotland. Modes of transmission of HCV, incubation of infection, and cofactors in the development of primary liver cancer were also considered.

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Methods

Patients

A total of 114 cases of histologically confirmed hepatocellular carcinoma (mean (SD) age: 69.2 (11.4) years; 90 male; 109 (95%) of whom were ethnically Scottish), presenting to the Royal Infirmary, Edinburgh between 1 January 1985 and 31 December 1994 were included. The cases were obtained from examination of biopsy and necropsy data (stored in the University of Edinburgh Pathology Department) and also International Classification of Diseases hospital admissions data.

The records of these patients were examined to identify the aetiology of HCC. Documentation of the histology of liver tissue surrounding the cancer, together with possible sources of transmission and duration of blood borne infectious hepatitis was made. The time of transmission of HCV infection was calculated from the year of first intravenous drug abuse or first blood transfusion; only these patients were used to calculate the range/median duration of infection. Serum samples had been stored at -70°C , from the time of diagnosis of HCC.

Serological markers of HBV infection were detected with standard assays (RIA, Abbott laboratories, Weisbaden, Germany).

Anti-HCV antibodies were detected by a second generation enzyme immunoassay (EIA, Abbot laboratories, Weisbaden, Germany) and also by third generation recombinant immunoblot assays (RIBA-3, Chiron, Emeryville, CA) for antibody to non-structural proteins 5-1-1 (NS4), c-100-3 (NS4), c33c (NS3), and core-associated antigen c-22-3.

A population of cirrhotic patients ($n=29$; 22 male; mean (SD) age: 52 (14.5) years), presenting during the same time period (1985–1994), was also examined. All had chronic hepatitis C infection; a significant proportion had previously been diagnosed as having 'cryptogenic' cirrhosis.

RNA extraction

Viral RNA was extracted from 0.5 ml of stored serum from each of the patients as previously described.²⁰ Briefly, serum samples were incubated at 37°C for 1.5 hours with 1 mg/ml proteinase K in the presence of 40 $\mu\text{g}/\text{ml}$ polyadenylic acid, 0.5% SDS, 0.1 M NaCl, 50 mM TRIS HCL (pH 8.0), and 1 mM EDTA. Nucleic acid was extracted successively with phenol and chloroform-isoamyl alcohol (50:1) and precipitated by the addition of one tenth volume of sodium acetate (pH 5.2) and two volumes of ethanol. The dried pellet was re-suspended in 25 μl of diethyl pyrocarbonate treated water.

HCV genotyping

RNA was reverse transcribed and amplified using nested primers matching conserved regions in the 5'-NCR.²¹ Product DNAs were cleaved with restriction enzymes Hae-III/Rsa-I and Mva-I/Hinf-I.²² The fragments were

separated by agarose gel electrophoresis using 4% metaphor agarose (FMC BioProducts, Rockland, ME). Phylogenetic comparisons of sequences in the conserved region of the genome confirm that the 5'-NCR can be used to distinguish the six major genotypes of HCV.²³ Separation of subtypes 1a and 1b was undertaken by the cleavage patterns resulting from digestion with Mvn-1.²⁰ In three cases direct nucleotide sequencing of PCR products was carried out.

Results

Of 114 patients with HCC, presenting to the Royal Infirmary, Edinburgh over a 10 year period, 80 (70%) stored serum samples were available for HCV testing and assessment of HCC aetiology.

Aetiology of HCC

HCV Ab serology was positive in 24 (21 male; mean (SD) age: 67 (12) years; all ethnically Scottish) cases (30% of tested samples); of whom two also had markers of current HBV infection (HBsAg positive), and a further six had markers of past infection (HBsAg negative; anti-HBs positive; anti-HBc positive). An additional two had a history of alcohol abuse (>40 units/week) for more than 10 years. Twenty of 24 cases were positive for HCV RNA by RT-PCR.

Examination of cases with negative HCV serology indicated that 13 (16%) were chronic carriers of the HBV virus (HBsAg positive); alcohol misuse was the sole risk factor in 16 (20%) cases; haemochromatosis in seven (9%); primary biliary cirrhosis in two (2.5%), and acute intermittent porphyria in two (2.5%). In 16 (20%) cases, the aetiology of liver cancer could not be determined either from the medical records or from serology (Fig 1).

HCV genotyping in HCC and cirrhotic populations

Genotyping of the virus was possible in 20 of 24 patients; it was 1b in 16; genotype 4 in three, and genotype 5 in one. In the cirrhotic population, a broader genotype distribution was demonstrated: genotype 1a: $n=7$; genotype 1b: $n=8$; genotype 2b: $n=3$; genotype 3a: $n=8$, and genotype 4: $n=2$. One cirrhotic patient was serum HCV RNA negative by RT-PCR (Table).

Risk factors for HCV transmission and duration of infection

Examination of risk factors for HCV transmission identified 15 patients who had had blood transfusions between 1940 and 1972 in Scotland; two of these had had more than one transfusion. One, had current infection with HCV, HBV, and HIV at diagnosis following injection of intravenous drugs between 1979 and 1989. A further eight patients (one of whom was a Jehovah's Witness) had no recognised risk factors for HCV infection. There

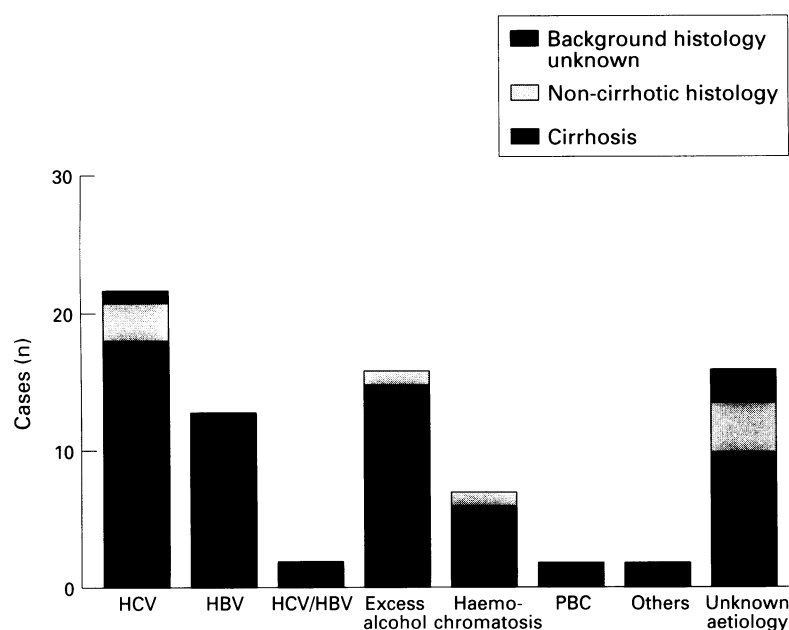


Figure 1: Aetiology of hepatocellular carcinoma (diagnosed between 1985 and 1994) in the 80 patients in whom stored samples of sera were available for anti-HCV testing. The bars are stacked to illustrate the background histology of the patients.

were no data available concerning the acquisition of HCV infection by covert percutaneous exposure of non-parenteral routes. The time from presumed HCV transmission to development of HCC ranged from 10 to 50 years (median 30) in the post-transfusion/intravenous drug misuse patients.

The range in duration of infection in the cirrhotic population was 10 to 40 years (median 19); this was a significantly shorter duration than in the HCC population ($p=0.0009$).

Histology of non-cancerous liver tissue in the HCC population

Cirrhosis preceded HCC in 20 of 24 HCV infected cases (83%). In two cases, HCC developed from a hepatitic liver, and in one (the patient had a lobectomy), the background histology of the tumour was normal (Fig 2).

In the whole HCC population, cirrhosis preceded HCC in 66 (82.5%) cases; other pathology was demonstrated in eight (10%),

and it was unclassified (because the tumour alone was biopsied) in six (7.5%).

Discussion

In this study, we have identified chronic HCV infection as a major risk factor for the development of HCC in a Scottish population, a finding already made in high risk areas of HCC, such as Japan, Italy, and Spain.⁴⁻⁹

There is a strong association between chronic HCV infection and hepatocarcinogenesis, through the pathway of chronic liver injury, regeneration, and cirrhosis; cirrhosis preceded HCC in 20 of 24 (83%) of our cases (Fig 2). In Western countries, the cancer commonly occurs late in life, mostly in cirrhotic nodules.²⁴⁻²⁵ Our study supports this finding; the patients were elderly and most were infected after receiving blood transfusions a median time of 30 years previously. This is a similar time period to that noted in a recent study from Spain, which found the interval between the date of blood transfusion and the diagnosis of HCC to be 26.8 (12.4) years.²⁶ However, in three of our cases, a tumour developed from a normal or hepatitic liver; further evidence for the persistence and replication of HCV genomes in primary liver cancer developing in the absence of cirrhosis or other risk factors.²⁷ In these cases, the mode of hepatocarcinogenesis is unclear; HCV lacks reverse transcriptase activity and is not thought to be associated with genomic integration, while little is known about the biological properties of HCV proteins. Certainly, the mechanism is different to hepatitis B virus (HBV). HBV is believed to be directly oncogenic either by HBV integration causing increased genomic instability or by transactivation of cellular genes.²⁸⁻³⁰

Cofactors to HCV seropositivity in the development of HCC were noted in four patients; either current HBV infection (two patients) or alcohol misuse (two patients). A further six patients had markers of past hepatitis B infection (HBsAg negative; anti-HBs, and anti-HBc positive). HBV may still be implicated in these cancers because its DNA can persist in both serum and liver of patients with serology of past infection.³¹⁻³² The relation

Comparison between HCV associated HCC population and HCV associated cirrhotic population

	HCV associated HCC	HCV associated cirrhosis	Significance
Number	24	29	
Age (y)*	67 (12)	52 (14.5)	$p=0.0002$
Sex (M:F)	21:3	22:7	
Years of diagnosis	1986-1995	1986-1995	
Mode of infection	RCC Transfusion: 15 IVDA: 1 Sporadic: 8	RCC Transfusion: 9 IVDA: 11 Haemophiliac: 3 Sporadic: 6	
Duration of infection (y)†	30 (10-50)	19 (10-40)	$p=0.0009$
Alcohol misuse	2	9	
Current HBV infection	2	1	
Past HBV infection	6	9	
Serum RT-PCR positive (HCV RNA)	20	28	
HCV genotypes (%)	Genotype 1b: 16 (80) Genotype 4: 3 (15) Genotype 5: 1 (5)	Genotype 1a: 7 (25) Genotype 1b: 8 (29) Genotype 2b: 3 (10) Genotype 3a: 8 (29) Genotype 4: 2 (7)	

(*mean (SD); †median (range)). In the cirrhotic population a wider distribution of genotypes was noted; however, this population was significantly younger and had a significantly shorter duration of HCV infection than the HCC population.

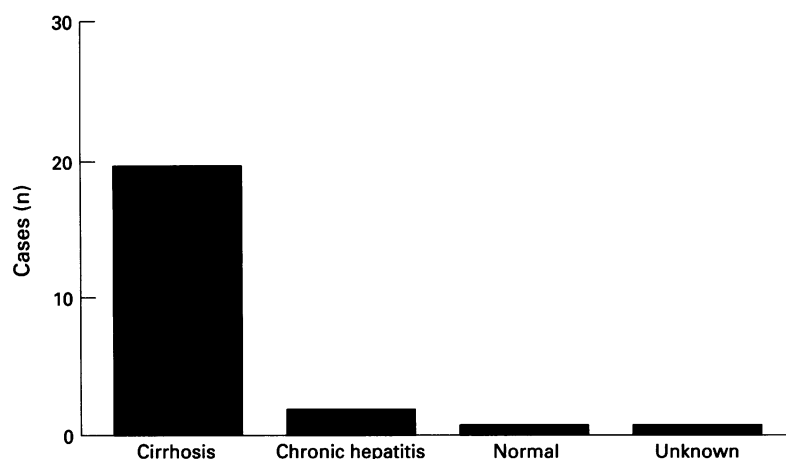


Figure 2: Histology of non-cancerous liver tissue. The background liver histology of the patients with positive anti-HCV serology is shown. The majority were cirrhotic (80%).

between the two viruses is unclear. In vitro, HCV core protein can suppress HBV expression and encapsulation³³; while clinical studies have suggested a reciprocal and inverse relation between HCV and HBV replication.³⁴ Further studies are required to determine whether there is synergy between the HCV genome and HBV DNA in the process of hepatic oncogenesis.

HCV genotype 1b was predominant (16 of 20 patients: 80%); this genotype has previously been reported to be responsible for the development of severe liver disease and the failure of interferon therapy.^{18 19 35 36} The dominance of this type contrasts both with the genotype distribution in current Scottish blood donors, (where type 1a forms the majority: 70–80%),¹⁴ and in our population of cirrhotic patients (only eight of 28 patients were infected with genotype 1b: 28%). The difference in epidemiology may be partly explained by the prevalence of type 1b in older patients and in those with a longer duration of infection (our HCC population was significantly older, and had been infected significantly longer than the cirrhotic population). The distribution of HCV genotypes among blood donors in Scotland 30 to 50 years ago is unknown; identification of patients who received blood transfusions containing genotypes other than 1b, and their clinical course, would provide a control group to the patients who developed cancer in this study. Unfortunately, it is not possible to identify these patients and the suspicion remains that the prevalence of genotype 1b in this HCC population represents the most prevalent genotype a median time of 30 years ago.

Genotype 4 is commonly associated with HCV infection in Egypt, other African countries, and the Middle East.³⁷ It has not been reported in Scottish or European blood donor populations. The six patients with genotype 4 and HCC had not visited any of these countries; however, all six were infected through blood transfusions in the 1940s and 1960s. Likewise, genotype 5 was also associated with HCC in one case, where a patient had received a blood transfusion in 1966, but had never travelled to the African continent. This genotype was originally found in South Africa; low frequencies have been reported in

the Netherlands, Australia, and Canada.³⁷ Currently, this is the only evidence for the association of genotypes 4/5 and primary liver cancer in the Western world.

In conclusion, these findings indicate that, in a population of Scottish patients, there is a strong association between chronic HCV infection, cirrhosis, and hepatocarcinogenesis. However, the study was unable to distinguish whether the high prevalence of genotype 1b in the HCC population reflects increased oncogenicity in itself, or simply a background epidemiology of the most prevalent genotype, a median time of 30 years ago. Larger, population based studies are required to confirm that HCV genotypes vary in their propensity to produce clinically significant liver disease; and to establish the role of cofactors and the host response in these processes.

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